Differential Heat Shock Tolerance and Expression of Heat-Inducible Proteins in Two Stored-Product Psocids

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ABSTRACT The recent recognition of psocids as a major concern in stored products and also the reemergence of heat treatment as a control tactic of stored-product insects led to the present investigation. The objectives of this study were to determine whether there are differences in heat shock tolerance of two species of stored-product psocids—*Lepinotus reticulatus* Enderlein (Trogiidae) and *Liposcelis entomophila* (Enderlein) (Liposcelididae)—and to determine whether heat shock proteins (HSPs) underlay such tolerance. Time–response bioassays were therefore carried out at increasing temperatures for both psocids. The lethal time $(LT)_{50}$ and LT_{95} estimates were correlated with the expression of heat shock proteins after exposure at the same range of temperatures for 30 min. The expression of HSP was determined through Western blot analyses using HSP 70 antibody. *Liposcelis entomophila* was more than two-fold more tolerant than *L. reticulatus* for nearly all of the range of temperatures (≥40.0°C). Expression of HSP 70 was not observed for either of the psocid species, but the expression of two low-molecular-mass heat-inducible proteins (HIPs; 23 and 27 kDa) was observed in *L. entomophila*. The expression of these small proteins was induced by exposure to higher temperatures, and the trend was particularly strong for HIP 27. In contrast, no expression of small heat-inducible proteins was detected in *L. reticulatus*, reflecting its higher susceptibility to heat treatments. The relatively high heat tolerance of *L. entomophila* might help explain its more common occurrence in grain stored in warmer regions of the world.

KEY WORDS heat stress, heat treatment, *Lepinotus reticulatus*, *Liposcelis entomophila*, heat shock proteins

Psocoptera (psocids) is a relatively small insect order containing several species adapted to live in stored bulk grains, food processing facilities, warehouses where processed food is stored, and urban retail stores and dwellings (Sedlacek et al. 1996, Baz and Monserrat 1999, Lienhard and Smithers 2002, Pascual-Villalobos et al. 2005). Psocids were generally regarded as scavengers and mold feeders of little importance as pests until the early 1990s (Sedlacek et al. 1996, Turner 1999, Kučerová 2002, Rees 2003), when their nuisance status changed to that of serious worldwide pests of stored products; this particularly applies to several *Liposcelis* species (Liposcelididae), including *L. entomophila* (Enderlein) and *L. bostrychophila* Badonnel, and *Lepinotus reticulatus* Enderlein (Trogiidae) (Sedlacek et al. 1996, Turner 1999). Psocids are a little-studied group, but recent reports indicate that their occurrence in large numbers compromises the quality of stored commodities they infest leading to eventual rejection of the commodities for export (Turner 1999, Rees 2003). Psocids are also allergens, causing discomfort and health problems among storage workers (Burgess et al. 1991, Mills et al. 1992, Baz and Monserrat 1999, Elston 1999).

The behavioral and physiological peculiarities of stored-product psocids seem to make their management more difficult. The short life cycle and parthenogenetic mode of reproduction of many species allow them to rapidly colonize new habitats (Sedlacek et al. 1996, Turner 1999). The likely movement of psocids out of the dry bulk grain environment to absorb atmospheric moisture at times of high ambient humidity probably limits fumigation efficiency by reducing their exposure to the fumigant in open-top silos (Rees 2003). In addition, the relatively long period of egg development, sometimes >15 d for *Liposcelis*, delayed hatching in the presence of phosphine, and phosphine resistance also compromise phosphine fumigation against psocids (Nayak et al. 2003b). Among stored-product protectants, pyrethroid insecticides are reported as relatively ineffective against *Liposcelis*

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spp., and even organophosphate and carbamate insecticides show limited efficacy against stored-product psocids (Nayak et al. 2003a, Wang et al. 2004). Novel insecticides, such as spinosad, also seem unable to provide effective control against stored-product psocids resulting in use of insecticide mixtures to increase efficacy to desirable levels (Nayak et al. 2005, Nayak and Daglish 2007).

Carbon dioxide-enriched atmospheres and heat treatments are possible alternative methods of managing stored-product psocids; however, resistance to carbon dioxide in psocids is a concern (Wang et al. 2000). Heat treatment recently reemerged as a potential control method for stored-product insects and is currently drawing interest, particularly as an alternative for structural fumigations with methyl bromide (Burks et al. 2000, Mourier and Poulsen 2000, Neven 2000, Tang et al. 2000, Roesli et al. 2003). The effect of temperature on the development and reproduction of *L. entomophila* was studied by Wang et al. (1998), but to date the mortality at extreme temperatures has not been studied except for the work of Beckett and Morton (2003). This study indicated higher vulnerability of stored-product psocids to high temperatures (45–50°C) than for stored-product beetle pests, but it did not include the key psocid pest species *L. entomophila.* Physiological changes caused by heat treatment, which are rooted in biochemical changes mainly in the form of production of heat shock proteins (HSPs) (Feder and Hofmann 1999, Neven 2000, Malmendal et al. 2006), have not been studied in stored-product psocids.

Heat shock proteins work as molecular chaperones that, under heat stress, may hold in situ the nascent or denatured proteins chaperoning them to the lysosome for degradation or helping them to refold after return to favorable temperatures (Hendrick and Hartl 1993, Feder and Hofmann 1999, Neven 2000). Most of the literature on insect heat shock proteins and thermotolerance focuses on the stress proteins in the 60–80-kDa range in a few insect species (Neven 2000; Mahroof et al. 2005a,b; Shim et al. 2006; Bettencourt et al. 2007; Sørensen and Loeschcke 2007). This HSP 70 family, as characterized by its molecular weight and function, is the best characterized and its role in thermotolerance in insects is well documented. Their effects are usually short-lived but potentially important for acute thermal stress as commonly applied in heat treatments for stored-product protection. The induction of HSPs is important not only as a potential deterrent of insect control (by heat treatment) but also may be useful in predicting geographical distribution and potential problems with stored-product psocids associated with variable and unpredictable temperature variation (Dahlhoff and Rank 2007).

The abundance of *L. entomophila* in recent trap catches in the central United States, particularly during the summer (Throne et al. 2006), is suggestive of a higher heat shock tolerance in this species with potentially higher expression of heat shock proteins. Throne et al. (2006) found as many as 400 *L. entomophila* per 8.9 by 12.7-cm cardboard refuge on the surface of wheat in steel bins in late summer. In their 2004 sampling study, *L. reticulatus* and *L. entomophila* were the only psocid species found infesting stored grain; in both the summer and fall, numbers of *L. entomophila* in refuges were always much higher than those of *L. reticulatus.* To test the hypothesis that *L. entomophila* has higher heat shock tolerance, time–response bioassays were carried out at increasing temperatures (37.5, 40.0, 42.5, 45.0 and 47.5°C) for *L. reticulatus* and *L. entomophila.* The estimates of lethal times for 50 and 95% mortalities (LT_{50} and LT_{95}) were subsequently correlated with the expression of heat shock proteins after exposure to the same range of temperatures for 30 min. The results are expected to help explain temperospatial distribution of these psocid species and are relevant to use of heat treatments for control of psocids in structures.

Materials and Methods

Insects. Cultures used in the study were started with insects collected during summer 2004 in wheat (*Triticum aestivum* L.) stored in steel bins at the USDA Grain Marketing and Production Research Center in Manhattan, KS. The laboratory colonies were maintained on a diet of 97% cracked hard red winter wheat, 2% Rice Krispies (Kellogg USA Inc., Battle Creek, MI), and 1% brewer's yeast (MP Biomedicals Inc., Solon, OH) (wt:wt; cracked wheat diet) in 0.473-liter glass canning jars covered with mite-proof lids. Cultures were maintained at 30°C, 75% RH, and 24-h scotophase (Opit and Throne 2008a,b). Only adult females were used in the experiments because *L. reticulatus* is parthenogenic and *L. entomophila* is not. Voucher specimens of *L. reticulatus* and *L. entomophila* used in this study were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under lots 181 and 182, respectively.

Time–Mortality Bioassays with Increasing Temperature. Adult female psocids of both species were subjected to time–mortality bioassays at the increasing temperatures of 37.5, 40.0, 42.5, 45.0, and 47.5°C (30°C was used as reference temperature for assessing natural mortality). These temperatures are representative of the temperature range observed in heat treatments of flour and feed mills (e.g., Mahroof et al. 2003, Roesli et al. 2003). Fifty insects were released in each petri dish (3.5 cm in diameter), whose walls were covered with Teflon PTFE (DuPont, Wilmington, DE) to prevent insect escape. Three replicates of this experimental unit were used for each combination of insect species, heat shock temperature, and length of exposure. The petri dishes containing insects were closed and placed on plastic waffle-type false floors that were placed in dark plastic boxes (26 by 36.5 by 15 cm) containing saturated NaCl solution to maintain 75% RH and 24-h scotophase (Greenspan 1977). The dark boxes containing the petri dishes with insects were placed in temperature-controlled chambers (model 136 VL, Percival Scientific, Inc., Perry, IA) set at the desired temperature of exposure. Temperature and humidity inside the chambers were monitored

with HOBO data recorders (Onset Computer, Bourne, MA); mercury thermometers also were used to verify the temperature readings within the chambers. Mortality assessment was carried out 24 h after the desired heat shock exposure, and psocids were considered dead if they were unable to walk after prodding with a fine brush. The mortality assessments were carried out at regular and independent exposure intervals (i.e., with independent replicates in time) preestablished after preliminary tests.

Determination of Temperature-Dependent Expression of HSPs. Adult female psocids of both species were exposed for 30 min to 30.0 (control treatment), 37.5, 40.0, 42.5, 45.0, and 47.5°C by using the same methods already described. After the heat shock exposure, the insects were immediately frozen at −80°C in an ultralow temperature freezer in which they were maintained until used. Frozen insect samples were homogenized in ice-cold 0.02 M phosphate buffer, pH 7.0, containing 0.2% (vol:vol) Triton X-100 (400 insects/250 μl; three batches of such samples were used for each combination of psocid species and temperature). The homogenates were centrifuged at 11,500 × *g* for 3 min at 4°C. The supernatants were used for total protein determination and analysis of HSP expression. Quantification of total protein in the crude insect extracts was carried out in triplicate by using the bicinchoninic acid assay method developed by Smith et al. (1985) but adapted for use with a V_{max} enzyme kinetic microplate reader (Molecular Devices, Menlo Park, CA) and bovine serum albumin as the protein standard (Sigma, St. Louis, MO), as described previously (Guedes et al. 1997).

Western Blot Analysis of HSPs. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of adult females of *L. entomophila* and *L. reticulatus* was carried out on 4–20% Tris-glycine precast gels, pH 8.6, by using a XCell II Mini-Cell gel apparatus (Novex, San Diego, CA). Samples standardized for the amount of protein were mixed with 2× SDS sample buffer (Invitrogen, Carlsbad, CA), heated for 5 min at 85°C in a water bath following Mahroof et al. (2005a) and loaded onto individual wells of the gel (120 μg protein/well for each psocid sample). Electrophoresis was carried out at 105 V for 2 h in 1× NU-PAGE MOPS SDS running buffer (Invitrogen). Molecular mass standards ranging from 20 to 120 kDa (MagicMark Western standard, Invitrogen), bovine brain HSP 70 (Sigma), and maize weevil (*Sitophilus zeamais* Motschulsky [Coleoptera: Curculionidae]) extract (25 adult insects/300 μl buffer; exposed to 45°C for 30 min and extracted using the same protocol for psocids) also were simultaneously run on each gel for determining the molecular mass of the psocid HSP (molecular mass standards) and serving as a double positive control for HSP 70 (bovine HSP 70 and maize weevil extract).

The proteins resolved on the SDS-PAGE were electrotransferred onto polyvinylidene difluoride membrane (pore size, 0.2 μm; Invitrogen) at 25 V for 1.5 h by using XCell II blot module (Novex) with 1× Novex Tris-glycine transfer buffer (Invitrogen) containing 20% (vol:vol) methanol. Immunodetection of HSP was carried out using WesternBreeze chemiluminescent anti-mouse kit according to the manufacturer's instructions (Invitrogen). Monoclonal anti-bovine brain HSP 70 antibody produced from mouse (IgG isotype, Sigma) and anti-mouse IgG antibody conjugated with alkaline phosphatase (Invitrogen) were used as the primary and secondary antibodies, respectively. The chemiluminescent reaction was identified by briefly exposing an X-ray film (Kodak X-Omat AR film, Eastman Kodak, New Haven, CT) to the membrane sandwich for 1 min. The film was developed and fixed using Kodak GBX developer and fixer (Sigma).

Quantification of HSP was carried out using a gel analysis densimeter and AMBIS Radioanalytic Imaging System (Ambis Systems, San Diego, CA). The densities from insect samples subjected to heat shock were normalized to the density of the representative HSP band quantified in the control treatment (insects exposed to 30°C). The objective of this procedure was to correct for between-gel variation in HSP detection. A similar normalization procedure was carried out for temperature-dependent HSP expression. Relative molecular masses of HSPs were estimated based on R_f values from linear calibration curves.

Statistical Analysis. Time–mortality bioassays with increasing temperature were subjected to probit analysis (PROC PROBIT, SAS Institute 2002). The selectivity ratio between both psocid species at each temperature was obtained by dividing the LT_{50} and LT_{95} of *L. entomophila* by the corresponding LT estimates for *L. reticulatus.* The 95% CL of these estimates were calculated following Robertson and Preisler (1992), and the LT values for the two species were considered significantly different ($P < 0.05$) if the confidence limits on the selectivity ratio did not include the value 1. Nonlinear regression analyses were carried out using temperature (°C) as the independent variable and the LT_{50} and LT_{95} estimates as the dependent variables, and also using these same estimates as independent variables and the expression of HSP as the dependent variable. The expression of HSP also was regressed against the heat shock temperature. All of the regression analyses were carried out using the curve-fitting procedure of TableCurve 2D (SPSS Inc. 2000).

Results

Time–Mortality Responses. The time–mortality results from exposure of adult females of the two species of stored-product psocids under investigation, after correction for natural mortality (<5%), showed low χ^2 values and high *P* values (<11.0 and >0.05, respectively), indicating the suitability of the probit model for fitting the time–response curves and consequently obtaining estimates of the mortality parameters LT_{50} and LT_{95} (Table 1). The values of LT_{50} and LT_{95} for both psocid species decreased with increasing temperature, indicating more rapid mortality with higher heat shock temperatures (Fig. 1). *Liposcelis entomophila* showed higher overall tolerance to heat treatments than *L. reticulatus* (>2× and 3× on average for

Table 1. Time–mortality responses for two psocid species subjected to heat-shock at increasing temperatures

Species	Temp (°C)	*n*	df	Slope ± SEM	LT_{50} (95% FL) (h)	Selectivity ratio at LT_{50} (95% CL)	LT_{95} (95% FL) (h)	Selectivity ratio at LT_{95} (95% CL)	χ^2	*P*
Lepinotus reticulatus	37.5	904	4	0.040 ± 0.004	49.69 (46.83–52.98)		90.92 (83.00–102.35)		4.56	0.21
	40.0	963	4	0.245 ± 0.022	7.10 (6.67–7.60)		13.83 (12.59–15.56)		5.73	0.22
	42.5	1,177	5	1.130 ± 0.107	2.38 (2.20–2.57)		3.84 (3.50–4.36)		10.87	0.05
	45.0	775	2	8.468 ± 0.679	0.51 (0.49–0.52)		0.70 (0.67–0.74)		4.33	0.11
	47.5	754	2	10.461 ± 0.812	0.44 (0.42–0.46)		0.60 (0.57–0.63)		3.00	0.22
Liposcelis entomophila	37.5	919	4	0.021 ± 0.002	34.11 (28.96–38.92)	0.69 (0.58–0.79)[a]	111.43 (100.19–126.88)	1.22 (1.03–1.42)[a]	1.51	0.82
	40.0	1,057	4	0.057 ± 0.004	14.99 (13.24–19.51)	2.11 (1.84–2.38)	43.52 (39.90–48.23)	3.15 (2.71–3.59)	5.00	0.29
	42.5	860	3	0.266 ± 0.045	2.88 (1.33–3.91)	1.21 (0.92–1.49)	9.06 (6.96–15.68)	2.36 (1.82–2.90)	7.05	0.07
	45.0	1,126	4	0.585 ± 0.041	2.69 (2.49–2.89)	5.31 (4.88–5.75)	5.51 (5.16–5.95)	7.85 (7.17–8.54)	3.76	0.44
	47.5	1,063	5	2.556 ± 0.196	1.25 (1.19–1.30)	2.83 (2.67–2.99)	1.89 (1.81–1.99)	3.16 (2.96–3.37)	3.21	0.67

[a] The LT values for the two species were considered significantly different ($P < 0.05$) if the confidence limits on the selectivity ratio did not include the value 1 (Robertson and Preisler 1992).

temperatures ≥40°C, respectively, based on the LT_{50} and LT_{95} selectivity ratios) (Table 1; Fig. 1). In addition, it takes longer to kill 50% of *L. reticulatus* at 37.5°C, but it is quicker to reach 95% mortality of this species at this temperature than for *L. entomophila.* This takes place because the time–mortality response curves for *L. entomophila* showed consistently lower slopes than those for *L. reticulatus* reversing the relative heat tolerance at LT_{95} compared with LT_{50} at 37.5°C. The lower slopes of the time–mortality curves of *L. entomophila*, particularly at the highest temperatures, indicates a higher heterogeneity of response to

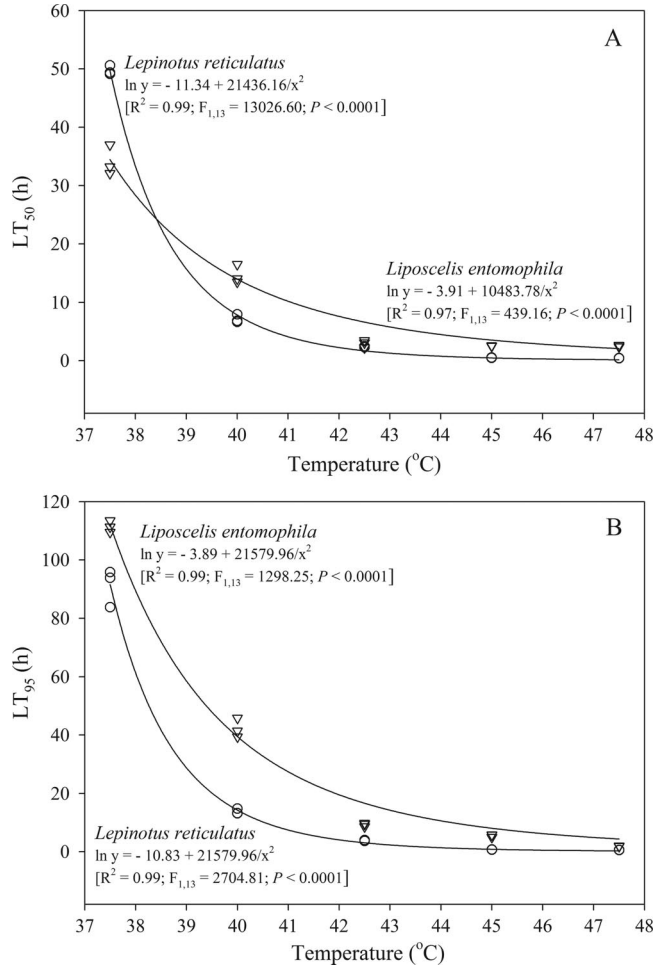

Fig. 1. Effect of heat shock temperature on the heat shock tolerance (based on the LT_{50} [A] and LT_{95} [B]) of two species of stored-product psocids: *L. reticulatus* (○) and *L. entomophila* (▽).

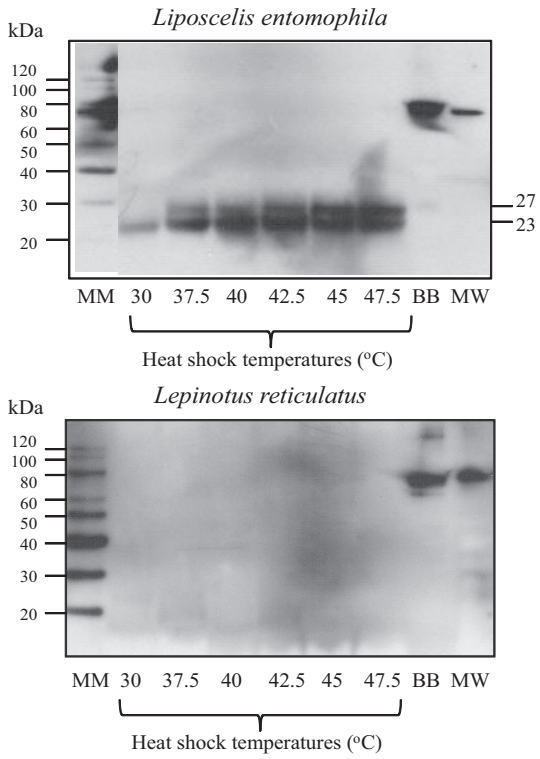

Fig. 2. Western blot analysis of heat shock proteins in two species of stored-product psocids after exposure for 30 min to the range of temperatures indicated in the bottom part of each panel. MM, molecular mass standard; BB, bovine brain heat shock protein 70 (positive control); MW, maize weevil heat shock protein 70 (positive control; 100 μg/lane). Lanes contain equal amounts of protein for psocids (120 μg per lane).

heat shock among individuals of this species compared with *L. reticulatus* (Table 1).

Expression of Heat Shock Proteins. The gel bands for the bovine brain HSP 70 and the maize weevil HSP, both used as a double positive control (Fig. 2), were within the expected range of molecular mass of HSP 70s on the Western blot analysis and indicate that there was no problem in our experiments and that the monoclonal anti-bovine brain HSP 70 antibody produced from mouse was successful for detecting HSP 70. This was expected because HSP 70s are highly conserved in different organisms and the HSP 70 antibody used here also has been successfully used in other insect species (Hendrick and Hartl 1993; Feder and Hofmann 1999; Mahroof et al. 2005a,b). In contrast, the monoclonal anti-HSP 70 antibody failed to recognize any conserved epitope of HSP 70 in either *L. entomophila* or *L. reticulatus* exposed to control (30°C) or heat-treated conditions (from 37.5 to 47.5°C) (Fig. 2), despite the concentrated insect extracts used (120 μg protein per well) and the highly sensitive chemiluminescent detection technique used here for detecting HSP 70 in the Western blotting analysis. Such failure to detect HSP 70 could be due to the lack of HSP 70 or a very low level of HSP 70 expressed in these psocid species, undetectable with the technique used. Curiously, unexpected reactivity of the anti-bovine HSP 70 monoclonal antibody resulted in protein bands of 27 and 23 kDa in adult females of *L. entomophila*, here referred to as heat-inducible protein (HIP) 27 and HIP 23, but not in *L. reticulatus* (Fig. 2). The reference to these small proteins as "heat inducible" rather than "heat shock" was used because more evidence (e.g., through their purification and characterization) is required to recognize them as belonging to any small HSP family and exhibiting chaperone role.

Relationship between HIP Expression and Heat Shock Stress in *L. entomophila*. The HIP bands of 27 and 23 kDa observed in the Western blots of *L. entomophila* were quantified and their levels of expression, based on the control temperature (30°C), were related to the range of heat shock temperatures in which they were generated. Regressions were significant ($P < 0.05$) for both small HIPs (sHIPs): HIP 23 and HIP 27 (Fig. 3). The relationship was particularly robust ($R^2 = 0.79, P < 0.001$) for the expression of HIP 27 with a steeper increase in its expression with an increase in heat shock temperature. The relationship for HIP 23 was not as robust and was more variable, although significant, with a smaller increase of expression than for HIP 27 ($R^2 = 0.49$, $P = 0.001$).

The levels of expression of both HIP 23 and HIP 27 from *L. entomophila* increased with the heat shock susceptibility of this species based on its LT_{50} and LT_{95} values determined at the heat shock temperatures of the current study ($P < 0.05$). Because the results with LT_{50} and LT_{95} were very similar, only the LT_{50} curves were presented (Fig. 4). The expression of HIP 23 showed smaller, although significant ($P < 0.05$), variation in expression, with both LT_{50} and LT_{95} of *L. entomophila* subjected to increasing heat stress (Fig. 4). HIP 27 showed only vestigial levels of constitutive expression and a three- to five-fold increase in expression with heat shock stress (Fig. 4), particularly during higher heat stress (at the lower LT_{50} and LT_{95} values; Fig. 4).

Discussion

Our study shows higher variability and tolerance to heat shock stress in the stored-product psocid *L. entomophila* than in *L. reticulatus.* HSP 70 was not detected in either of the psocid species, but *L. entomophila* expresses small heat-inducible proteins (23 and mainly 27 kDa) sharing a common epitope with HSP 70, which were not detected in *L. reticulatus* and seems to underlay the higher heat shock tolerance of *L. entomophila.*

A higher heat shock tolerance of *L. entomophila* was expected based on its abundance in recent trap catches in the central United States during summer and fall (Throne et al. 2006). Haines and Pranata (1982) found *L. entomophila* to be the most frequently encountered stored-product psocid in tropical Indonesia. Therefore, higher expression of HSP 70 also was

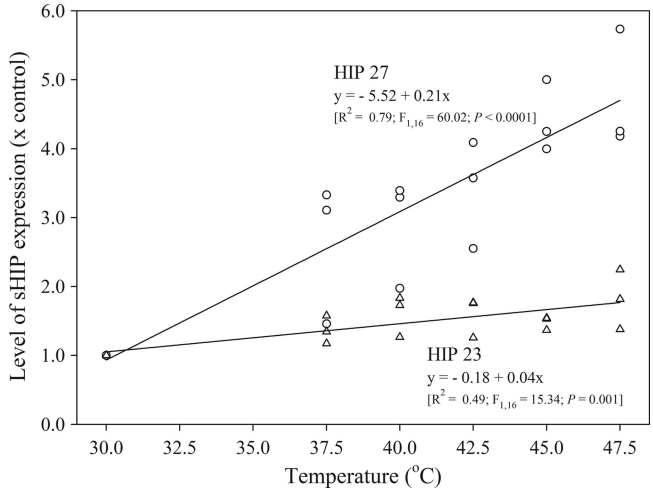

Fig. 3. Effect of heat shock temperature on the expression of small heat inducible proteins HIP 27 (○) and HIP 23 (▽) in the stored-product psocid *L. entomophila*.

expected with increased heat shock stress in this species compared with *L. reticulatus.* We indeed observed higher tolerance to heat shock stress in *L. entomophila* than in *L. reticulatus.* Although the patterns of heat-inducible protein expression were consistent with this finding, the HSP 70 family does not seem involved as chaperones providing protection against acute thermal stress in the psocid species studied. Such a role seems to fall with the small heat-inducible proteins HIP 23 and mainly HIP 27 in *L. entomophila*, as reported in other species (Kim et al. 1998, Suzuki et al. 1998, Feder and Hofmann 1999).

Differential heat shock tolerance among the stored-product psocids *Liposcelis bostrychophila* Badonnel, *L. decolor* Pearman, and *L. paeta* Pearman was earlier reported by Beckett and Morton (2003). Therefore, the differential heat shock tolerance between *L. entomophila* and *L. reticulatus* is not surprising, especially considering the differences that exist in their abundance and seasonal distribution in the central United States (Throne et al. 2006). Despite this differential tolerance, heat shock treatments seem effective against stored-product psocids based on limited studies (Beckett and Morton 2003; this study). However, insect acclimatation did not occur in our study, but it may take place with some modalities of heat treatment where insects may have up to a few hours to adapt and may lead to a different response from what we observed.

The variation of heat shock response among individuals of *L. entomophila* also is suggestive of the potential existence of populations of this species exhibiting differential susceptibility to heat shock stress. Because variation in heat shock response was already

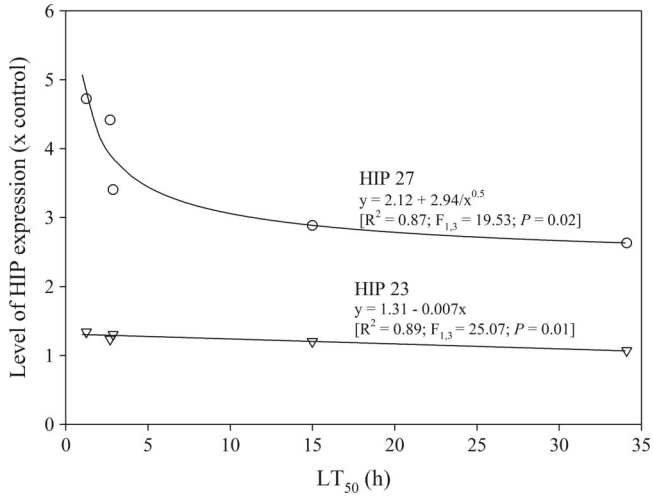

Fig. 4. Effect of the heat shock susceptibility (based on the LT_{50}) on the level of expression of small heat-inducible proteins HIP 23 (○) and HIP 27 (▽) in the stored-product psocid *L. entomophila*.

reported in the willow leaf beetle *Chrysomela aeneicollis* Schaeffer (Chrysomelidae) (Dahlhoff and Rank 2007), a species associated with an environment of variable and unpredictable temperature variation, the same may be true for other species. Although the stored-product environment does not have variable and unpredictable temperature, the wild sources of *L. entomophila* infestation may afford such conditions. In addition, altitudinal and latitudinal clines are likely to afford environmental conditions for differential susceptibility to heat shock stress among populations of widely distributed species (Sørensen and Loeschcke 2007), such as *L. entomophila* (Sedlacek et al. 1996, Turner 1999).

Lepinotus reticulatus was more susceptible to heat shock stress and did not express any detectable level of HIPs. Although consistent, these results are surprising due to the ubiquitous distribution of heat shock proteins in living organisms, especially HSP 70 and its association with acute thermal stress (Hendrick and Hartl 1993, Feder and Hofmann 1999, Neven 2000, Sørensen and Loeschske 2007). *Lepinotus reticulatus* may, however, produce small heat-inducible proteins similar to *L. entomophila*, but these proteins do not share homology with HSP 70 at the particular epitope recognized by the (mammalian) monoclonal antibody used in our study. Further molecular studies are necessary to elucidate the relationship between the heat-inducible proteins identified in our study and those found in other organisms.

Liposcelis entomophila provides an interesting contrast to *L. reticulatus* because of its higher relative tolerance to heat shock stress, as expected, and its higher heterogeneity of response. In addition, this species also did not express detectable levels of HSP 70 under heat stress, but it did express two different small proteins induced by heat stress (i.e., HIP 23 and HIP 27). The nondetection of HSP 70 expression in these species may result either from very low levels of expression of HSP 70 in psocids, below the threshold levels of detection for the technique used in our study, or from the lack of reaction between psocid HSP 70 and the monoclonal antibody used, which was developed against a mammalian HSP 70. Small heat shock proteins have been recognized as protective heat stress agents in other living organisms (Kim et al. 1998, Suzuki et al. 1998, Feder and Hofmann 1999), but their possible role in thermal tolerance in insects has been barely investigated (Frydenberg et al. 2003, Mahroof et al. 2005a; Morrow et al. 2006).

A small protein also was detected with monoclonal anti-bovine brain HSP 70 antibody in the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebronidae), although it was not inducible by heat stress (Mahroof et al. 2005a). The authors suggested that this 24-kDa protein observed in young larvae of the red flour beetle may have resulted from the breakdown products of HSP 70. It might have been so, but then the expression of additional 24-kDa protein bands would likely have occurred in other developmental phases of the same insect subjected to the same extraction procedure, which was not the case. Alternatively, the 24-kDa protein detected may in fact be another fully functional protein of small molecular mass with sufficiently high homology to the bovine HSP 70 to allow its detection. We favor this last explanation in the case of the sHIPs detected in *L. entomophila* because they seem functional, and one of them, HIP 27, was particularly strongly expressed with heat stress. In addition, no vestigial sign of HSP 70 bands were visible, which would be expected if the sHIPs were the result of its breakdown. Despite this, the possibility of the sHIPs being the result of HSP 70 breakdown products cannot be refuted at the present time.

Clinal variation in the expression of sHSPs has been reported in insect species (Dahlhoff and Rank 2007, Sørensen and Loeschcke 2007). This fact lays credence to the potential ecological role of thermal adaptation by clinal or other geographic variation in insect populations (Sørensen and Loeschcke 2007). Therefore, the causal relationship between heat shock stress and expression of sHIP in *L. entomophila* is likely to be a useful tool in surveying potential variation in heat shock resistance in this species and for assessing the potential for its evolution in the applied context of the heat treatments used for controlling stored-product insects. The high heterogeneity of response of adult females of *L. entomophila* to heat shock temperatures is suggestive of enough variability for further selection, which may eventually lead to the evolution of populations of this species with reduced susceptibility to heat stress. However, high fitness cost is usually associated with the expression of heat shock proteins (Feder and Hofmann 1999, Chown and Nicolson 2004, Sørensen and Loeschcke 2007), which may delay the evolution of heat shock resistance in stored-product psocids, and this deserves future research. In addition, the lack of detectable expression of heat-induced HSP protein in *L. reticulatus* and the expression of sHIPs (and not HSP 70) with likely homology with HSP 70 in *L. entomophila* also warrants further study preferentially encompassing more psocid species and populations.

Our study suggests that greater heat tolerance in *L. entomophila* might lead to its more common occurrence in grain stored in warmer regions of the world, compared with occurrence of *L. reticulatus.* Indeed, work by Haines and Pranata (1982) seems to support this. Our results also suggest that heat treatments should be efficacious for both of these psocid species. However, the greater heat tolerance and higher heterogeneity of response to heat shock in *L. entomophila* might facilitate its development of thermal tolerance or resistance to heat treatments in the grain storage facilities.

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References Cited

Baz, A., and V. J. Monserrat. 1999. Distribution of domestic Psocoptera in Madrid apartments. Med. Vet. Entomol. 13: 259–264.

Beckett, S. J., and R. Morton. 2003. The mortality of three species of Psocoptera, *Liposcelis bostrychophila* Badonnel, *Liposcelis decolor* Pearman and *Liposcelis paeta* Pearman, at moderately elevated temperatures. J. Stored. Prod. Res. 39: 103–115.

Bettencourt, B. R., C. C. Hogan, and M. Nimali. 2007. Polyglutamine expansion in *Drosophila:* thermal stress and Hsp70 as selective agents. J. Biosci. 32: 537–547.

Burgess, I., M. Coulthard, and J. Heaney. 1991. Scalp infestation by *Lyposcelis mendax.* Br. J. Dermatol. 125: 400–401.

Burks, C. S., J. A. Johnson, D. E. Maier, and J. W. Heaps. 2000. Temperature, pp. 73–104. *In* Bh. Subramanyam and D. W. Hagstrum [eds.], Alternatives to pesticides in stored-product IPM. Kluwer, Boston, MA.

Chown, S. L., and S. W. Nicolson. 2004. Insect physiological ecology: mechanisms and patterns. Oxford University, Oxford, New York.

Dahlhoff, E. P., and N. E. Rank. 2007. The role of stress proteins in responses of a montane willow leaf beetle to environmental temperature variation. J. Biosci. 32: 477–488.

Elston, D. M. 1999. What's eating you? Psocoptera (book lice, psocids). Cutis 64: 307–308.

Feder, M. E., and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61: 243–282.

Frydenberg, J., A. A. Hoffmann, and V. Loeschcke. 2003. DNA sequence variation and latitudinal associations in *hsp23, hsp26* and *hsp27* from natural populations of *Drosophila melanogaster.* Mol. Ecol. 12: 2025–2032.

Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Natl. Bur. Stand. A 81: 89–96.

Guedes, R.N.C., K. Y. Zhu, B. A. Dover, and S. Kambhampati. 1997. Partial characterization of phosphotriesterases from organophosphate-susceptible and -resistant populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Pestic. Biochem. Physiol. 57: 156–164.

Haines, C. P., and R. I. Pranata. 1982. Survey on insects and arachnids associated with stored products in some parts of Java, pp. 17–48. *In* N. C. Teter and A. S. Frio [eds.], Proceedings of the 5th Annual Workshop on Grains Post-Harvest Technology Progress in grain protection. Southeast Asia Cooperative Post-Harvest Research and Development Programme-Laguna, Philippines.

Hendrick, J. P., and F.-U. Hartl. 1993. Molecular chaperone functions of heat-shock proteins. Annu. Rev. Biochem. 62: 349–384.

Kim, R., K. K. Kim, H. Yokota, and S.-H. Kim. 1998. Small heat shock protein of *Methanococcus jannaschii,* a hyperthermophile. Proc. Natl. Acad. Sci. U.S.A. 95: 9129–9133.

Kučerová, Z. 2002. Weight losses of wheat grains caused by psocid infestation (*Liposcelis bostrychophila:* Liposcelidae: Psocoptera). Plant Prot. Sci. 38: 103–107.

Lienhard, C., and C. N. Smithers. 2002. Psocoptera (Insecta)–World catalogue and bibliography. Natural History Museum, Geneva, Switzerland.

Mahroof, R., Bh. Subramanyam, J. E. Throne, and A. Menon. 2003. Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. J. Econ. Entomol. 96: 1345–1351.

Mahroof, R., K. Y. Zhu, and Bh. Subramanyam. 2005a. Changes in expression of heat shock proteins in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in relation to developmental stage, exposure time, and temperature. Ann. Entomol. Soc. Am. 98: 100–107.

Mahroof, R., K. Y. Zhu, L. Neven, Bh. Subramanyam, and J. Bai. 2005b. Expression patterns of three heat shock protein 70 genes among developmental stages of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Comp. Biochem. Physiol. 141A: 247–256.

Malmendal, A., J. Overgaard, J. G. Bundy, J. G. Sørensen, N. Chr. Nielsen, V. Loeschcke, and M. Holmstrup. 2006. Metabolic profiling of heat stress: hardening and recovery of homeostasis in *Drosophila.* Am. J. Physiol. Reg. Integr. Comp. Physiol. 291: R205–R212.

Mills, J. T., R. N. Sinha, and C. J. Demianyk. 1992. Feeding and multiplication of a psocid, *Liposcelis bostrychophilus* Badonnel (Psocoptera: Liposcelididae) on wheat, grain screenings, and fungi. J. Econ. Entomol. 85: 1453–1462.

Morrow, G., J. J. Heikkila, and R. M. Tanguay. 2006. Differences in the chaperone-like activities of the four main small heat shock proteins of *Drosophila melanogaster.* Cell Stress Chaperones 11: 51–60.

Mourier, H., and K. P. Poulsen. 2000. Control of insects and mites in grain using a high temperature-short-time (HTST) technique. J. Stored Prod. Res. 36: 309–318.

Nayak, M. K., and G. J. Daglish. 2007. Combined treatments of spinosad and chlorpyrifos-methyl for management of resistant psocid pests (Psocoptera: Liposcelididae) of stored grain. Pest Manag. Sci 63: 104–109.

Nayak, M. K., P. J. Collins, and R. A. Kopittke. 2003a. Residual toxicities and persistence of organophosphorus insecticides mixed with carbaryl as structural treatments against three liposcelidid psocid species (Psocoptera: Liposcelididae) infesting stored grain. J. Stored. Prod. Res. 39: 343–353.

Nayak, M. K., P. J. Collins, H. Pavic, and R. A. Kopitke. 2003b. Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). Pest. Manag. Sci. 59: 1191–1196.

Nayak, M. K., G. J. Daglish, and V. S. Byrne. 2005. Effectiveness of spinosad as a grain protectant against resistant beetle and psocid pests of stored grain in Australia. J. Stored Prod. Res. 41: 455–467.

Neven, L. G. 2000. Physiological responses of insects to heat. Postharv. Biol. Technol. 21: 103–111.

Opit, G. P., and J. E. Throne. 2008a. Effects of diet on population growth of the psocids *Lepinotus reticulatus* Enderlein and *Liposcelis entomophila* (Enderlein). J. Econ. Entomol. 101: 616–622.

Opit, G. P., and J. E. Throne. 2008b. Population growth and development of the psocid *Lepinotus reticulatus* at constant temperatures and relative humidities. J. Econ. Entomol. 101: 605–615.

Pascual-Villalobos, M. J., A. Baz, and P. Del Estal. 2005. Occurrence of psocids and natural predators on organic rice in Calasparra (Murcia, Spain). J. Stored Prod. Res. 41: 231–235.

Rees, D. 2003. Psocoptera (psocids) as pests of bulk grain storage in Australia: a cautionary tale to industry and

researchers, pp. 59–64. *In* P. F. Credland, D. M. Armitage, C. H. Bell, P. M. Cogan, and E. Highley [eds.], Advances in stored product protection. CAB International, Wallingford, United Kingdom.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

Roesli, R., Bh. Subramanyam, F. Fairchild, and K. Behnke. 2003. Trap catches of stored-product insects before and after heat treatment of a pilot feed mill. J. Stored. Prod. Res. 39: 521–540.

SAS Institute. 2002. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC.

Sedlacek, J. D., P. A. Weston, and R. J. Barney. 1996. Lepidoptera and Psocoptera, pp. 41–70. *In* Bh. Subramanyam and D. W. Hagstrum [eds.], Integrated management of insects in stored products. Marcel Dekker, New York.

Shim, J.-K., D.-O. Jung, J.-W. Park, D.-W. Kim, D.-M. Ha, and K.-Y Lee. 2006. Molecular cloning of the heat-shock cognate 70 (*Hsc70*) gene from the two-spotted spider mite, *Tetranychus urticae*, and its expression in response to heat shock and starvation. Comp. Biochem. Physiol. 145B: 288–295.

Smith, P.K.R., R. I. Krohn, T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76–85.

Sørensen, J. G., and V. Loeschcke. 2007. Studying stress responses in the post-genomic era: its ecological and evolutionary role. J. Biosci. 23: 447–456.

SPSS Inc. 2000. TableCurve 2D user's guide, version. 5. SPSS Inc., Chicago, IL.

Suzuki, T. C., D. C. Krawitz, and E. Vierling. 1998. The chloroplast small heat-shock protein oligomer is not phosphorylated and does not dissociate during heat stress in vivo. Plant Physiol. 116: 1151–1161.

Tang, J., J. N. Ikediala, S. Wang, J. D. Hansen, and R. P. Cavalieri. 2000. High-temperature-short-time thermal quarantine methods. Postharv. Biol. Technol. 21: 129–145.

Throne, J. E., G. P. Opit, and P. W. Flinn. 2006. Seasonal distribution of psocids in stored wheat, pp. 1095–1103. *In* I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, D. Sundfeld, J. P. Santos, E.J.D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L.O.F. Bortolini, M. R. Sartori, M. C. Elias, R.N.C. Guedes, R. G. Fonseca, and V. M. Scussel [eds.], Proceedings of the 9th International Working Conference on Stored Product Protection. ABRAPÓS, Campinas, Brazil.

Turner, B. D. 1999. Psocids as pests: the global perspective. Int. Pest Control 41: 185–186.

Wang, J.-J., Z.-M. Zhao, and L.-S. Li. 1998. Studies on bionomics of *Liposcelis entomophila* (Psocoptera: Liposcelididae) infesting stored products. Entomol. Sinica 5: 149–158.

Wang, J.-J., J. H. Tsai, Z.-M. Zhao, and L.-S. Li. 2000. Development and reproduction of the psocid *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) as a function of temperature. Ann. Entomol. Soc. Am. 93: 261–270.

Wang, J.-J., W.-X. Cheng, W. Ding, and Z.-M. Zhao. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and *L. entomophila.* J. Insect Sci. 4: 23–28.

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